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Phytochemical screening of a medicinal plant: Lavandula *stoechas* (Lamiaceae)

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Abstract
With an aim of developing the aromatic and medicinal plants of Morocco, we were interesting during this study in the characterization and the phytochimic identification of Lavandula *stoechas* collected in the area of the average Atlas.
Lavandula *stoechas* (Lamiaceae) is a species largely used in traditional medicine for its biological properties allotted primarily to polyphenols. In order to identify the latter in the various parts of the plant (leafs, stems, fruits, roots), we used tests phytochimic based on the reactions of colouring and the chromatographic analyses (CCM). Several extractions with solvents of different polarities were carried out. The results of the extraction of polyphenols by maceration show that water is the best solvent of extraction, followed by the ethyl acetate. In the same way the tests confirm the presence of polyphenols, the flavonoids, tannins, saponins, the reducing compounds, sterols and triterpenes and the cardiac glycosides.

Keywords: Lavandula stoechas, phytochemical, CCM, polyphenols, non-polyphenolic compounds.

Introduction
During decades spent, the public interest for the natural therapies increased considerably in the industrialized countries [1].
The annual import reported a worldwide pharmaceutical plant is the average to 400,000 tons worth 1.224 million. The international trade is dominated by only some countries [6].
The use of these plants as drugs is a tradition of long time. It practically goes up at the beginning of humanity [2].
With its geographical location (true crossroads between Europe and Africa and the Mediterranean and the Atlantic), and with the diversity of its climate and its habitats, Morocco shelters particularly varied natural vegetation, as well by its structure and its aspect, for example: The diversity of the species. The floristic wealth of the country is mainly related to the ecological heterogeneity of its biotopes, the rich flora of the country is mainly due to the heterogeneity of its ecological habitats, it is estimated 5211 species and sub-species distributed in 155 families and 981 genera, and the number of medicinal and aromatic plants (MAP) in Morocco is about 800 species [3].
Morocco is therefore a major producer and exporter of fresh plants, essential oils (EO) and concrete.
These (EO) Moroccan is very known on the international market and more particularly in Europe and in the USA. Principal oils essential manufactured in Morocco are: rosemary, the white wormwood, lavender, thyme, petrols of citrus fruits and the myrtle [7].
These aromatic and medicinal plants, which are exploited in form dried or extracts, represent an important tank of products having various activities and which can have multiple commercial applications in perfumery, food industry and in the pharmaceutical and biomedical fields [4]. In Morocco, the lavender exists in a natural state in several regions, in particular in the Anti-Atlas, the High Atlas, the Average Atlas, the Mediterranean coastline and Rif Eastern [13]. For a long time we know the healing and antiseptic properties of this species. It accelerates the healing of burns and wounds and it calms inflammation due to insect bites. It is also recommended to treat scabies and lice [14].
In this context, we are interested to study the species: Lavandula *stoechas* (Lamiaceae), of the area of the Average Atlas. The aim of our work is to demonstrate the richness of these plant secondary metabolites and determine their biological properties. To do this, our study includes two parts, the first is the

phytochemical order based primarily on the qualification of phenolic compounds. It also relates to the diagnosis and the separation of the main compounds by the use of chromatographic techniques.
The second part is devoted to an evaluation of the antioxydant activity of these metabolites with respect to free radical DPPH and antibacterial, antifungal tests in order to determine the effectiveness of these compounds against the micro-organisms which one will publish later on.

Material and Methods

Plant material

1) Choice of the site

The plant Lavandula *stoechas* was collected in the area of the Average Atlas during the year 2015 at the period of its flowering, it comprises approximately twenty-eight species, which are in most Mediterranean origin [8, 9] which is generally presented in the form of a sub-shrub.
This last is with persistent stem and sheets, it can reach a length of 1 meter, narrow green pale, extends from the major bluish gray to the green with brown pale, flowers of color blue-purple. Other varieties are with white and pink flowers [10, 11].
The inflorescence described by a dense spike, shortly stalked, with small purple flowers. The spike is crowned with several bracts petaloids violets. There are four stamens and a tubulous lipped corolla [12].

2) Preparation of the samples

The plant was collected in the month of May 2015 to the period of its flowering. The samples (leaves, roots, stems, fruits) are rinsed and dried in a dry place, aired safe from the light during two weeks and crushed using a crusher.
The identity of this plant was confirmed by the laboratory of botany of the university Ibn Tofail.
The chemical tests of characterizations were carried out on the powders prepared before hand from each body of the plant using the reagents of characterization [15].
The principle of the chemical characterization of the active molecules of Lavandula *stoechas* consists in revealing by qualitative analysis the extracts resulting from the different organs.

3) Preparation of the extracts

The extraction is the separation of the active parts of plants by using selective solvents by means of standard procedures. The products thus obtained starting from plants are relatively complex mixtures of metabolites, at the liquid or semi-solid state or (after elimination of solvent) in the form of dry powder, and are intended to be used by oral or external way.
Those understand classes of preparations known under the name of decoctions, infusions, fluidextracts, dyeings, extracted (semi-solid) or the powder extracts [5].

a) Aqueous extract

consist in introducing vegetable powder 1g into 20 ml of boiling water which one lets infuse during 15 minutes. Then, one filters and one rinses with a little warm water so as to obtain 20 ml of filtrate.

b) Extract acetatic

consist has to introduce 1g of vegetable equipment into 20 ml of the ethyl acetate then one lets it macerate during 24 hours.

4) Screening phytochimic

Screening phytochimic is a means to highlight the presence of the groups of chemical families present in a given drug.

A-Preliminary Tests

The tests of characterization are based partly on the qualitative analysis, either on the formation of insoluble complexes by using the reactions of precipitation, or on the formation of coloured complexes, by using reactions of colouring [16].

Alkaloids

The tests are carried out by precipitation reactions with the Dragendorff reagent. 1 ml of each extract is taken into test tubes and 5 drops of Dragendorff reagent, the appearance of an orange precipitate, is found to reveal the presence of alkaloids [17].

Reducing compounds

Their detection consists in treating 1 ml del' extracts with 2 ml from distilled water and 2 ml of Fehling's solution then the tubes are heated with the bath Marie with 40°C. A positive test is revealed by the formation of a precipitate red-brick [18].

Glycosides cardiac

2 ml of chloroform are addition has 1 ml of the extract, the appearance of a brown-reddish colouring after the addition of H_2SO_4 indicates the presence of the cardiac glycosides (36).

Polyphenolic Substances

Tannins

The presence of tannins is demonstrated by adding to 1 ml of each extract 1 ml of water and 1 to 2 drops of fecl3 solution diluted 1% The appearance of a dark green or blue green color indicates the presence of tannins. The appearance of a dark green color indicates the presence of catechic tannins.
The appearance of a blue green color indicates the presence of gallic tannins [18].

Flavonoids

To 5 ml of extract to be tested, add 1 ml of iso-amyl alcohol, some magnesium chips and a few drops of hydrochloric acid (HCl), the appearance of a pink or red coloration indicates the presence of flavonoids [17].

Saponins

In a series of 10 test tubes numbered 1 to 10, respectively, introduce 1, 2, 3...; 10 ml of the analytical solution to be prepared by decoction in an aqueous, aqueous-alcoholic or infusion medium. Then adjust the volume of each tube to 10 ml with distilled water and stir each tube along the length of the tube for 15 seconds at 2 stirring per second. Let stand 15 min and measure the height of the foam produced in each tube. The foam index (I) is calculated by the following formula:
$I = 1000 / N$
N is the number of the tube where the foam height is equal to 1 cm [17]

Sterols and triterpenes

An ether extract is prepared from 1 g of vegetable powder in 20 ml of ether for 24 hours. Sterols and triterpenes are demonstrated by adding 1 ml of $CHCl_3$ to the residue of 10 ml

of the evaporated macerate.
The solution obtained is divided into two test tubes and then 1 to 2 ml of concentrated H_2SO_4 is added to the bottom of one of the tubes and the other will serve as a control. The formation of a brownish or violet red ring has the contact zone revealed their presence [16].

Coumarins
Dry evaporate 5 ml of ethereal extract under the hood and add 2 ml of hot water to the residue. Share the solution between two test tubes. Then add 0.5 ml of ammonia diluted to 25% and observe the fluorescence under UV 366 nm. The appearance of fluorescence in the tube, where ammonia was added indicates the presence of coumarins [34].

Free quinones
One gram of dry and crushed vegetable equipment is placed in a tube with 15 to 30 ml of oil ether. After agitation and a rest of 24:00, the extracts is filtered and concentrated with the rotavapor. The presence of free quinones is confirmed by the addition of a few drops of NaOH 1/10, when the aqueous phase transfers with the yellow, red or purple [30].

Compounds cyanogenic
Three grams of fresh vegetable equipment are wet with a few drops of chloroform ($CHCl_3$) in a test tube, where is inserted an impregnated strip of filter paper of sodium picrate. The tube is then placed at the bain-marie with 35°C during 3 hours. A turn with the red of the strip indicates the presence of the compounds cyanogenic [30].

Mucilages
To introduce 1 ml of decocted into a test tube and to add 5 ml of pure alcohol, obtaining a flocculent precipitate per mixture indicates the presence of mucilages [34].

Essential oils
1g of Plant material was introduced into 10 ml of dichloromethane then the extract was evaporated dry. The residue was then dissolved in 3 ml of ethanol. Then, the solution was evaporated dry again. The feeling of a scented odor indicates the presence of essential oils [31].

Extractable substances by water
Place in a flask 1 g of powder and 20 ml of distilled water, then make a decoction for 15 minutes and allow cooling for 20 minutes. Filter on filter paper and weigh an empty capsule (n) and put the filtrate into this capsule, then evaporate to dryness and weigh the capsule with the residue (n').

Extractable substances by water = (n' – n) x100 [32]

Free anthracene derivatives
To introduce into a test tube of the chloroformic extract (1ml), to add diluted ammonia (1ml) and to agitate. The more or less intense presence of red colouring indicates free anthraquinones [34].

Anthracene derivatives compounds
-*O*-Heterosides
Extract hydrolysate (5 ml) and stir with chloroform (5 ml) in a separatory funnel without emulsion formation. Then remove the organic phase, introduce it into a test tube and retain the aqueous phase and Add diluted ammonia (1ml) and shake. The presence of anthraquinones is revealed by the red coloring. If the reaction is negative or weakly positive, look for the reduced genome *O*-heterosides: Take 5 ml of hydrolysate, add 3-4 drops of 10% FeCl3, then heat for 5 minutes on a water bath and cool under the stream of water and stirring with chloroform (5 ml), it is necessary to remove the organic phase and introduce it into a test tube. Then dilute ammonia (1 ml) is added and the mixture is stirred. In the presence of oxidation products of anthronols or anthrones, red coloring is more intense than previously [34].

-*C*-Heterosides
To take again the aqueous phase which was kept in the funnel separating in a test tube and add on this phase of distilled water (10 ml), of ferric chloride with 10% (1ml) and to carry to the bain-marie during 30 minutes. Then it is necessary to cool under water current and to agitate with chloroform (5 ml). Remove the chloroform phase and add dilute ammonia (1 ml) and stir. A more or less intense red color indicates the presence of *C*-heterosides genes [34].

B-Thin layer chromatography (TLC)
The realization of a separating analysis; namely the thin layer chromatography (TLC) completing the characterization by phytochemical screening. The support, used in this study is a silica gel plate (20 × 20 cm, 60 F254), developed by different gradients migration solvents. After drying, the chromatograms were found either in the visible or UV/366 nm with or without suitable developers (table 1).

Table 1: Extraction solvents and Chromatography Thin Layer System

Secondary metabolites	Extraction solvents	Migration Solvents	Revelation reagents
Tannins (33)	Acetone	Ethyl acetate / Methanol / H_2O (40 : 8 : 5)	Ferric chloride / acetic acid / water (2: 2: 96)
Saponins (19)	Methanol	Chloroform/ Methanol/ H_2O (100: 13,5: 4)	Antimony trichloride
Anthraquinones (35)	Methanol	Ethyl acetate / Methanol / H2O (81 : 11 : 8)	Potassium hydroxide
Coumarins (37)	Chloroform	Ethyl acetate / Toluene (10 : 93)	ammonia NH3
Flavonoids	Methanol	Ethyl acetate / Methanol / H2O (100: 13,5: 10)	5% aluminium chloride in mixture methanol / water (1: 1)
Terpenoids (19)	Hexane	Benzene	Antimony trichloride

Results

A- Identification with color reaction

Table 2: combining the results of the identification of chemical compounds

Compounds	Extraction solvent	Leaf	Stem	Fruit	Root
Polyphenols	Aqueous	++++	++++	++++	++++
	Ethyl acetate	++	+	+	+
Tannins ($FeCl_3$)	Aqueous	++++	++++	++++	++++
	Ethyl acetate	++	+	+	+
Gallic tannins	Aqueous	++++	+	+	-
	Ethyl acetate	-	-	-	-
Catechic tannins	Aqueous	+	+	+	++
	Ethyl acetate	-	-	-	-
Alkaloids	Aqueous	-	-	-	-
	Ethyl acetate	-	-	-	-
Reducing compounds	Aqueous	+	+	+++	+++
	Ethyl acetate	++++	+++	++	++
Flavonoids: Antchocyanes	Aqueous	-	-	-	+
	Ethyl acetate	-	-	-	-
Flavonoids: Leucoanthocyanins	Aqueous	-	-	-	+
	Ethyl acetate	-	-	-	-
Flavonoids: Cyanidin	Aqueous	Flavones	Flavonols	Flavones	Flavonols
	Ethyl acetate	-	-	-	-
Cardiac glycosides	Aqueous	++++	++++	++++	++++
	Ethyl acetate	++++	++	+++	++
Sterols and Terpenes	Ether	++++	+++	++++	++++
Coumarins	Ether	++	+	+	+
Quinones	ether Petroleum	++++	+++	++++	++++
Cyanogenic	Chloroform	+++	+++	+++	+++
Mucilages	Aqueous	-	-	+	-
Essential Oil	Dichloromethane	+++	+++	+++	-
Free anthracene derivatives		-	-	-	-
O-heteroside		++++	++++	++++	++++
C-heteroside		++	++	++	++

++++: Strongly positive; ++ (+): Fairly positive; +: Slightly positive; - : Negative

According to table (2) above

The polyphenols and the simple tannins are present in greater quantities in the different parts of the plant for the aqueous extract and in average quantities for the acetate extract.

For Gallic Tannins: Important presence in leaves, traces in stems and fruits and absence in roots for aqueous extract and total absence in the different parts for the acetate extract.

For catechic tannins: an average amount in the roots, low in the leaves, stems and fruits for the aqueous extract and total absence in the different parts for the acetate extract.

For anthocyanins and leucoanthocyans: they exist with a small quantity only in the roots for the aqueous extract and absence in the different parts for the acetate extract.

The cyanidin reaction confirms the presence of flavonols in the stems and roots and flavones in the leaves and fruits for the aqueous extract and absence for the acetate extract in the different parts of the plant.

For reducing compounds: they are found in large quantities in fruits and roots and in small quantities in leaves and stems for aqueous extract and a high abundance in leaves and stems and average in fruits and roots for extract Acetate.

For alkaloids: there is a remarkable absence in the different parts for the 2 extracts.

The cardiac glycosides are with a great quantity in the various parts of the plant and the 2 extracts.

According to the got results, one can conclude that best the output of extraction of polyphenols, tannins (simple, gallic, catechic), flavonoids is obtained by aqueous maceration, whereas a acetatic maceration confirms a presence of the reducing compounds, cardiac glycosides are marked in great quantity by 2 solvents.

For the sterols and terpenes, a strong presence is observed in the different parts of the plant, whereas the coumarins are found in a small amount and this is confirmed by TLC.

For the quinones, there is a strong presence in the different parts of the plant.

The cyanogenic compounds are present with an average quantity in the different parts of the plant.

The mucilages are found only in fruits and with a small quantity.

Essential oils are on average amount in leaves, stems and fruits and absence in roots.

There is also an absence of free anthracene derivatives in the different parts of the plant, on the other hand there is a large quantity of *O*-heterosides and an average quantity of *C*-heterosides.

Table 3: Table combining the results of the identification of saponins

Solvent / Compounds	Aqueous Leaf	Aqueous Stem	Aqueous Fruit	Aqueous Root
Foam Index	250	142.85	250	111.11

According to the got result, one observes higher values in the sheets and fruits that in the stems and roots.

Table 4: The results of the identification of substances extractable by water

	Weight of empty beaker P1 (g)	Beaker weight after evaporation P2 (g)	P2-P1(g)	Percentage of substances extractable by water (%)
Leaf	49,3759	49,4438	0,0679	6,79
Stem	46,9762	47,0497	0,0735	7,35
Fruit	47,8814	47,9618	0,0804	8,04
Root	46,9845	46,9936	0,0091	0,91

According to the table so above, the percentage of the extractable substances by water is high in the sheets, stems and fruits and very weak at the roots.

B- Chromatographic Test Results

Phytochemical screening by color formation does not provide information on the nature of chemical molecules, so for further confirmation chromatographic tests are used (see Table 5).

Tableau 5: TLC results

Phytochemical Constituents	Leaf		Stem		Fruit		Root	
Tanins	Rf 0,961	Colour Rose	Rf 0,961	Colour Rose	Rf 0,961	Colour Rose	Rf -	Colour -
Iridoides	0,960 0,823	Green Green	-	-	0,980	Green	-	-
Saponins	0,903 0,692	Violet Violet	0,923 0,692	Violet Violet	0,903 0,692	Violet Violet	0,600	Violet
Anthraquinones	0,960 0,921 0,686	Red Green Red	0,921 0,882	Red Green	0,725	Red	0,600	Green
Coumarins	0,568 0,294	Pink Pink	0,961	Pink	0,961	Pink	0,627 0,529	Pink Pink
Flavonoids	0,945 0,763 0,581	Red Green Green	0,945	Red	0,927 0,818 0,581	Red Green Green	-	-
Sterols and triterpenes	0,900 0,820	Brown Brown	0,900 0,820 0,760	Brown Brown Brown	0,900 0,820 0,760	Brown Brown Brown	0,811 0,773	Brown Brown

The chromatographic analysis was made with an aim of separating different the metabolites present at the level as of extracts. This study related to the sheets, the stems, the fruits and the roots of the plant Lavandula *stoechas.*

The table above confirms the results got by colouring of the various molecules except for simple tannins that it was found that traces by reaction of colouring.

The examination of the CCM obtained watch that only tannins are represented by only one compound, whereas the other families of molecules account for 2 or 3 compounds with different frontal reports.

Discussion

In general, the presence of the chemical families detected for Lavandula *stoechas* in our study is confirmed by the work of Baptista *et al*, concerning polyphenols and flavonoids [20], by Ezzoubi *et al* for tannins, catechic tannins, Flavonoids and sterols [21], Teixeira *et al* for polyphenols, flavonoids and terpenes [22] and Jeffrey *et al* for flavonoids [23].

These results are comparable to those obtained in studies on Lavandula *officinalis* by Shafaghat *et al* [24], who confirmed the presence of tannins, flavonoids, the same on Lavandula *dentata* and Lavandula *angustifolia* by Abdelhady *et al* [25], which marked the presence of polyphenols.

The study of Lavandula *bipinnata* by Vidya *et al* [26] confirmed the presence of saponins, polyphenols, tannins and Lavandula *Antineae* by Krimat *et al* [27] for flavonoids, tannins, saponins and triterpenes.

Studies of Lavandula *latifolia* by Balakrishnan *et al* [28] detected flavonoids, phenols, saponins and tannins and Lavandula *x intermedia* by Blažeković *et al* [29] for polyphenols.

The phytochemical study carried out for this plant has shown results which are confirmed with other work carried out by authors, namely the presence of certain chemical families, whereas the absence of other families can be explained by a difference at the level of several geographic, physicochemical or biological parameters such as; The difference of the harvesting site including the environment of the plant or the genetic aspect between the plants or mutations at the level of the genes for a genetic adaptation.

Conclusion

Lavandula *stoechas* is among the aromatic and medicinal plants widely used in the pharmaceutical and biomedical fields

A qualitative phytochimic study followed of a confirmation by thin layer chromatography was prepared for the various parts of the plant for a characterization of chemical substances likely to be exploited on several scales (pharmaceutical, food, cosmetic…). The extraction of these compounds is a paramount stage for the valorization of these active ingredients; it depends on the method and suitable solvent.

The study proved that water maceration is the best technique for the identification and characterization of polyphenolic metabolites by comparing with ethyl acetate and, moreover, what was confirmed by the work Previous years.

The variation in the polyphenol content as a function of the part of the plant is not too significant, higher grades were marked in all parts except the roots.

To make good use of this natural substance, we will use a quantitative estimate of these metabolites for the next component.

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